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Controlled Ecological Life Support System

Higher Plant Flight Experiments

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Prepared for Ames Research Center Under Contract NCC 2-136



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SUMMARY

A panel of plant scientists was convened at NASA's Ames Research Center, Moffett Field, California July 13-15, 1983, to make recommendations on spaceflight experiments relating to use of higher-plant food crops in Controlled Ecological Life Support Systems (CELSS). Discussions were held to determine what research is needed to utilize higher plants effectively in space-farming systems and to develop recommendations for experimental approaches to meet these needs.

Research needs fell into two general categories: 1) physical parameters pertaining to nutrient, water, and gas exchange to plants, and 2) biological parameters affecting plant physiological functions.

Physical parameter concerns included:

- 1) Water and nutrient delivery through solid media.
- 2) Liquid transport to and from roots in hydroponic and/or aeroponic systems.
- 3) Oxygen and carbon dioxide solubility and diffusion in liquid and solid media.

Biological parameter concerns included:

- 1) Seedling establishment, involving root penetration into growing media, and seed coat shedding.
- 2) Orientation of roots, stem, and leaves to maintain plant productivity.
- 3) Flower initiation, poller transfer, and fertilization.
- 4) Accumulation of edible biomass.
- 5) Apical dominance.
- 6) Production and exchange rates for carbohydrate protein, oxygen, carbon dioxide, and water.

Studies of physical problems concerning plant life support were given highest priority as they were recognized to affect the implementation of all biological experiments.

The size, flight time, and environmental requirements for different experiments were discussed. The panel felt that certain experiments could be undertaken in the presently available small PGU's (volume approximately $0.01~\text{m}^3$). This would include many physical parameter tests and experiments utilizing small seedlings or excised plant parts. However, studies involving intact food-plant species, particularly productivity studies, will require a growing area on the order of a cubic meter or more. Some experiments could be undertaken in periods as short as 2 days but others would require 2 and possibly 3 months of growing time in space. The requirements for environmental control also will vary greatly. Many physical-parameter studies will require little more than temperature control, but most biological studies will require careful regulation of CO2, H2O, and CH4 to maintain constant levels in the plant growing area.

Hardware requirements were not a specific focus of this meeting since this topic was discussed at length at an earlier NASA workshop held April 21-22, 1983 at Ames Research Center. A summary of this April meeting is included as Appendix A of this document. Panelists agreed on the need for construction of plant growth units that could be readily modified to fit separate experiments. It was suggested that principal investigators should be directly involved in hardware design and construction and that prototype growth units should be provided to the principal investigators during experiment development.

The use of panels of plant scientists to coordinate experiment development by individual principal investigators was discussed.

The value of continuous interaction of the CELSS program with NASA's Space Biology program was emphasized and the development of plant growth hardware that could be utilized by both programs was encouraged.

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INTRODUCTION

Long-term plans in NASA's spaceflight program include the launch and construction of a space station that will be maintained in orbit for 10 or more years. Such a station would be manned by a crew of 8-12 people and be periodically supplied by shuttle vehicles. Calculations from previous studies (Olsen, 1983) predict that if this station remains in orbit for more than three years, the cost of producing food and oxygen for life support with space-grown plants will be less than the cost of continuous resupply from Earth. Thus, one of the goals of NASA's space program is to develop recycling plant-growing systems to supply at least a portion of the food and oxygen needs for human life in a space craft. This has been termed CELSS--Controlled Ecological Life Support Systems.

The current structure of CELSS research is divided into three interrelated parts: 1) food production with concomittant O2 and CO2 exchange, 2) waste processing, and 3) control management. The "food and O2 production" facet has been subdivided further into three separate approaches: utilization of photosynthetic algae, utilization of photosynthesis in higher plants, and utilization of nonphotosynthetic microbial food production either through chemically-synthesized substrates or substrates available in the waste stream. "Waste processing" will handle or consume the inedible parts from the production processes as well as human waste. The "control management" facet will ensure a life-sustaining balance between the inputs and outputs of production and consumption processes.

The needs and complexity of undertaking a higher-plants-CELSS at first appear to have many obstacles, but research from controlled environments and previous spaceflight experiments indicates that such a goal is within reach. The CELSS program will incorporate data obtained in experiments of the ongoing Space Biology program to provide basic information on plant responses to the weightlessness environment and to identify potential problems that need to be solved. A coordinated approach with a logical progression of experiments is paramount for success in developing the CELSS system. The July 1983 workshop was convened with this need in mind. Early sessions of the meeting were devoted to outlining recommendations on parameters needing study, after which experiment development was discussed along with the compatibility of present and planned plant growth units with specific studies.

Previous Higher Planc Workshops

The overall research needs for higher-plant production in CELSS were outlined in previous NASA workshops (e.g., Mason and Carden, 1982; Fabricant, 1983). These meetings emphasized the need for establishing the productivity of selected crop species in completely controlled environments and determining the problems associated with deficiencies and contaminant build-up in tightly recycled systems.

Two workshops were held in 1979-1980 to recommend higher plants species for consideration as space crops and to outline what research is needed to grow plants successfully in closed, recycling Priorities were given to species that provide high-caloric and /or high-protein foods. Recycling of minerals and vitamins, while being no less essential, was given little priority because of the ease of providing these substances from Earth with little payload effect. Eight species were recommended for primary consideration: wheat, rice, potato, sweet potato, soybean (or other bean spp.), peanut, lettuce, and sugar beet; six more species were suggested for limited-scale study: taro, winged bean, broccoli, strawberry, onion, and pea (Tibbitts and Alford, 1982). A later CELSS workshop at Ames Research Center, CA (July 1982) recommended that study be concentrated on three of these higher plant species -- wheat, potatoes, and beans -- to focus research efforts and obtain baseline information for evaluating the general usefulness of plants in CELSS.

The design of growth chambers and modules for housing plant experiments was discussed in several previous NASA workshops, including a meeting in April 1983 at Ames Research Center, CA. The results of the 1983 workshop are summarized in Appendix A of this document.

Purpose of This Workshop

This workshop was convened to propose spaceflight experiments that would support the research needs of higher plant use in CELSS. The NASA staff requested that the participants recommend experiments that could be undertaken during the presently-scheduled space shuttle flights, and during future flights, including the development of a manned space station. Thus, plant experiment recommendations were discussed for the available small plant growth units (PGU's), for new units with additional environmental control, and for large units with greatly increased environmental control that would be developed for future flights.

Workshop participants consisted of scientists that have been, or are currently participating in NASA research programs with plants. Several individuals directly involved with NASA Space Biology experiments were invited to participate to provide input on the transfer of basic information obtained in the Space Biology program and to promote an intergrated effort for experiment-hardware development.

PARAMETERS NEEDING STUDY

Previous experimentation from spaceflights has shown that higher plants will grow in space. For example, seeds can germinate and plants can develop roots, stems, and leaves under weightlessness (Ward et al., 1963: Krikorian ad Steward, 1978; Cowles et al., Therefore, the panel proceeded under the assumption that basic metabolic processes such as photosynthesis and respiration will function in space. Whether these processes function at rates comparable to earth-based plants is not known. Questions of this sort ultimately will be answered through productivity studies However, previous experimentation also during spaceflight. suggests that there can be many problems with plant growth in These problems need to be examined and controlled in order space. that plants can be exploited effectively for food production, water regeneration, and CO_2/O_2 exchange in space. Specifically, previous research has revealed a number of physical problems associated with providing needed water, mineral nutrients, and physical support for the plants (Brown and Chapman, 1982; et al., 1982). Problems with certain biological factors such as establishment of seedlings and orientation of shoots and roots also exist (Johnson and Tibbitts, 1968; Lyon, 1968; Cowles et al., 1982). Hence these subjects were the focus of discussions and recommendations during these meetings.

Physical Parameters

Water and Nutrient Delivery: Useful procedures for delivery of water and nutrients to plants under weightlessness were cited as a major concern for upcoming CELSS spaceflight testing. Because gravity-dependent movement of water cannot occur under weightlessness, other mechanisms of water movement must be employed.

The panel emphasized the need for studying both solid media and hydroponic (soilless) culture systems for growing plants. Solid media will have use in early experimentation phases of CELSS for ease of maintaining satisfactory growing conditions, but hydroponic approaches using thin films of water or misting systems most likely will be required in an operational CELSS to reduce the system's overall weight and to simplify regulation and control in the recycling of elements.

Capillary movement of water in solid porous media has been utilized effectively in short-term flight experiments (Brown and Chapman, 1982), and should be studied further with different types of materials that would have usefulness for supporting plant growth. Employment of wicking delivery systems also were cited for investigation.

Pressurized spray or misting systems should be evaluated in detail. Water droplet size and coalescence properties in the absence of gravity may present problems in these culture systems. Study of nutrient uptake and exchange at root surfaces also should be considered; this might include concerns for removal of any toxins accumulated at the root surface. Maintaining an adequate supply and exchange of nutrients may be particularly difficult if large amounts of liquid cling to the roots. Design and engineering of systems for collecting and recycling water and nutrients supplied to the root systems should be initiated.

Gas Exchange: Oxygen evolution and carbon dioxide uptake are two of the major contributions of photosynthesizing plants to a CELSS. Physical properties of gas movement in weightlessness should be known before adequate biological assessments can be made. This should include rates of oxygen and carbon dioxide diffusion through liquid and solid growing media and exchange between these media and the atmosphere. Once solubility and diffusion rates are determined, then solution, water-film, and aeroponic systems should be evalulated to determine how to provide adequate gas exchange at the root surface with each system. This may involve either adaptations providing additional aeration of the solution or development of procedures to ensure rapid replacement of the liquid supplied to the roots.

Rates of oxygen and carbon dioxide diffusion in the air around the leaves also were cited as a concern, but panelists agreed that this should be satisfied by the atmospheric agitation and exchange required in any operational CELSS. Hence this subject was given little emphasis regarding experimental recommendations.

Biological Parameters

Seedling Establishment: Recently-flown Space Biology plant experiments (Brown and Chapman, 1982; Cowles et al., 1982) have identified two problems in seedling establishment that should be studied in CELSS experimentation. These problems include: 1) difficulties for radicles from germinating seeds to penetrate a solid medium, and 2) the inability of cotyledons to shed their enclosing seed coat. Both problems have been encountered in Earth-based studies when seeds were germinated on media surfaces with no physical restriction around them. Flight experiments should be undertaken to determine the extent of problems for each

CELSS candidate species with radicle penetration and/or seed coat shedding following germination. If needed, procedures should be developed to provide physical resistance or containment of seeds to permit effective seedling establishment.

Orientation: Data from past spaceflights and numerous notizontal clinostat studies indicate that orientation problems will exist in weightlessness (Hoshizaki et al., 1966; Lyon, 1968; Brown et al., 1974). Therefore, the orientation of shoots and roots needs to be studied carefully during spaceflight to examine what effects, if any, this might have on plant productivity and cultural techniques.

Shoot growth, which usually is negatively gravitropic, must be studied to determine if light can be used as an orienting stimulus in the absence of gravity. The growth of each candidate species should be investigated under irradiance levels utilized for food production to establish whether effective orientation can be obtained.

Similarly, root growth of each of the selected species should be examined. Roots have been seen to protrude above the growing medium during spaceflight experiments and horizontal clinostating tests (Cowles et al., 1983; Hoshizaki, 1983). If restriction of overall plant productivity results from such random directional growth, systems should be devised to contain the roots and minimize the problem.

It is likely that leaf epinasty and associated elevated ethylene evolution will occur in some higher plants during spaceflight, particularly with dicot crop species (Johnson and Tibbitts, 1968; Leather et al., 1972). Experiments should be developed to ascertain whether leaf epinasty and /or elevated ethylene evolution cause growth reductions. If reductions are found, follow-up experiments should be conducted to study procedures for minimizing negative effects.

Flowering and Fertilization: Many of the selected crop species will require flower initiation, flower development, pollination, and fertilization, both for production of edible biomass and reproduction. Transfer of pollen from anthers to stigmas, growth of the pollen tube to effect fertilization, and embryo development should be studied in each of the appropriate species under weightlessness. Although studies of this sort would normally require intact flowering plants, it is conceivable that these processes could be examined using excised shoots or buds in short-duration tests. Ambient ethylene concentration should be carefully monitored in these studies because of its known effects on flower and fruit abortion, floral sex expression, and fruit maturation.

Although propagation may be provided through sterile, microculture procedures, it is felt that seed reproduction should be verified as an alternate mechanism of maintenance of the species.

Accumulation of Edible Biomass: Efficient carbohydrate and protein storage in edible plant organs and the normal development of these organs are crucial for the effective use of higher plants in space farming. Yet there is no evidence that vegetative organs, such as potato tubers, or reproductive propagules, such as seeds of wheat or soybeans, develop and accumulate carbohydrates and proteins normally under weightlessness. The specific growth and development of edible organs of each of the proposed crops should be studied in detail. For example, requirements for tactile stimuli for "undergound" food storage organs such as sweet potato roots, white potato tubers, and peanut, could be studied, particularly in proposed soilless-culture systems.

Levels of ethylene should be monitored in these studies when possible because of known regulation of tissue enlargement by this plant hormone.

Apical Dominance: Effects of weightlessness on apical dominance in the proposed crop species should be studied. Maintenance of healthy main shoot development may be crucial to high production of edible biomass in species where seeds are utilized for food. For example, the extent of tillering in cereal crops appears to be related to apical dominance controls. Excessive tillering of the species in confined growing spaces would most likely have negative effects on overall seed production.

Plant Productivity: A very important phase of space experimentation for CELSS will be to determine whether plant productivity in a space-farming system is comparable, or hopefully better, than that found in Earth-based systems. All aspects of productivity should be evaluated in these experiments, including: amount of edible biomass produced, proportion of inedible biomass to edible biomass, oxygen evolution, carbon dioxide consumption, and water regeneration. However, these studies cannot be attempted until sufficiently large plant growth units are available to sustain plants to maturity. These units must be capable of controlling all major environmental variables (viz. light, temperature, humidity, and CO₂) for the complete growth cycle of each plant species.

Estimates of large-scale production from space farming should be possible using single or limited-number plant production tests in spaceflight experiments. Small-scale flight test results could be correlated to large-scale, solid-stand control tests on the ground.

Initial productivity experiments could be undertaken in weightlessness. However, if productivity is significantly less than that in comparable ground-based systems, then application of artificial gravity through centrifugation should be explored as a means of enhancing production.

EXPERIMENT DIRECTION

Scheduling

No rigid priorities were established for the experiment scheduling, tut panelists emphasized the need for early research on physical parameters, because control of these physical factors would be required for successful conduct of most biological-parameter experiments. Also, some physical-parameter tests could be undertaken in presently available PGU's and could be conducted without plant systems, thereby eliminating the need for lighting and greatly simplifying environmental control requirements. Biological-parameter tests employing small seedlings or plant parts also could be accommodated in the presently available FGU's. instance, pollination and fertilization could be studied using flower buds that open during flight, or tuber formation in potatoes could be studied in the axillary buds of single leaves excised from mature plants. It should be possible to undertake short-rerm, plant part studies under much lower light levels than those required for whole plant studies. It is conceivable that experiments which normally would require high light levels might be accommodated in available low-light PGU's using sugar-supplemented growth media and sterile-cultured plants.

Hardware

The available plant growth units (PGU's) can be utilized for several of the recommended experiments. However, these units would have considerably greater use if modified with the addition of gas-exchange controls or provisions for continuous atmosphere replacement from a supply cylinder.

The low light intensities in the existing PGU's remain an impass to experiments requiring prolonged growth of plants. To some extent, light level is inherently restricted by the small size of the unit, hence experiments requiring higher irradiance levels may have to be delayed until larger chambers become available.

Panelists agreed that it would impossible to build a "universal" plant growth unit and that a high degree of flexibility to accommodate a wide variety of experimental requirements should be considered in the design of any future PGU's.

Hardware development on growth units for large-scale productivity studies should be initiated and designs should be developed that can be modified to fit available space in the different flight craft that evolve. This hardware must be designed to permit continuous plant growth for at least three months to carry the crop species to maturity. In contrast to the present PGU's which provide approximately 0.01 cubic meters volume, large-scale production units will require volumes on the order of 1.0 cubic meter or more.

Development of controlled-environment units that could be accommodated in free-flying "space platforms" also should be considered as possible step in this experiment program.

The meeting participants emphasized the need for having a number of plant growth modules made available for investigators during experiment development to obtain needed baseline response data in flight hardware. In addition, payload specialists should have access to plant growth units as training devices.

Scientist Interactions

The participants expressed concern for the need of involving a greater number of biological scientists in each experiment to provide quality assurance in experiments and to facilitate needed improvements in design and production of hardware.

The appointment of panels to assist in the development of each experiment was encouraged. Some participants felt that these panels should play a major role in the design of the space experiments along with outlining the test protocol to be followed; however, no general agreement was reached on this.

It was recommended that whenever possible the investigators should assume the responsibility for developing hardware. The hardware could be constructed by the principal investigator through a local subcontract. Biological instrument manufacturers might be contacted as potential contractors for hardware development.

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APPENDIX A

NASA-CELSS WORKSHOP ON EQUIPMENT

REQUIREMENTS FOR PLANT EXPERIMENTS IN SPACE

APRIL 1983

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The following is a summary of the conclusions reached by this workshop held at the NASA Ames Research Center, Moffett Field, CA, from April 21-22, 1983.

- 1. There was unanimous agreement that there is a great and pressing need for botanical experiments in space. Specific examples of experiments which need to be performed can be found in reports of several previous committees, and will not be discussed here in length. In general, the experiments fall into three categories. First, there are experiments to test the involvement of the Earth's 1-g gravity on morphogenetic processes such as root and shoot orientation, development of lignin in cell walls, or ability of plants of take up nutrients and transpire normally. Secondly, there is a great need for exploratory experiments to assess the role of gravity in the biochemistry and cytology of cells. Finally, we need to determine the optimal conditions for growing plants in space, if plants are ever to be a real part of any space station system.
- 2. At this time, the priority is to have vehicles which will permit the maximum frequency of botanical space experiments, even if this means that the vehicle will not be as sophisticated and flexible as desired. There would be great advantages to having one or more pieces of apparatus which could be used by a variety of experimenters without lengthy modification, since only under such circumstances will we be able to attract scientists to do the needed space botanical experiments.
- 3. The presently available units--the plant growth unit (PGU), the Heliflex apparatus, and the European Biorack module--are all valuable, but none is sufficient to permit frequent, meaningful experiments to be conducted with plants in the light.

4. We suggest that the first priority should be to modify the PGU to make it suitable for a series of plant experiments. deficiencies of the PGU are three-fold; lack of accessibility of the plants during flight, lack of a circulating, controlled atmosphere, and uneven light intensities. Modification to permit removal of the plants during flight is already underway, and should greatly improve the unit. We recommend the following major changes in the unic. First, the two end units should be removed and replaced with instrumentation units. The remaining four units would then have more uniform illumination. The unit must be adapted to have a circulating air system. We recommend that it not be a closed system, but involve flow-through of cabin air. This would need to have CO2 kept within some limits (350-3000 ppm) with a passive buffer system, some general control of humidity (perhaps obtained simply by allowing the plants to maintain a certain RH if the flow rate was low) and a system to remove volatiles such as ethylene or other hydrocarbons from the intake air (and perhaps from the efflux air as well). A second modification which we would suggest would be a video-tape recorder unit set to take time-lapse photographs. A third, and lower priority would be a system to allow one to add nutrients to the plants during the spaceflight. This chamber would have certain distinct disadvantatges; only small plants could be used, only "shade plants" would have their photosynthesis saturated at the light intensities available (120 umoles/s m²), no 1-g centrifuge control wauld be possible, and monitoring of environmental parameters such as CO2 and H2O would not be possible. is more than compensated by the fact that a modified chamber could be ready with only a short delay, that it would allow the number of experiments to be conducted in space to increase greatly, and that it would allow us to do the many needed exploratory experiments so that when more sophisticated chambers are ready, the correct experiments could be flown.

- 5. The second priority would be the construction of a new, 2-locker plant growth unit (PGU-2). The basic specifications would be similar to that of the PGU. A light intensity of 120 umoles/s m² would permit sufficient light to saturate photosynthesis of Temperature would be held at 25° C +1° C. shade plants. unit would have circulating air, with control of both CO2 and humidity, and removal of the possible volatile substances such as ethylene and isoprene which might interfere with plant growth. unit would have the ability to monitor the plants visually. principal difference between PGU-2 and the PGU would be the presence of a centrifuge as part of the equipment of this unit. The problem with most of the previous space botany experiments is that it has not been possible to eliminate the possibility that any ef.ects seen in the plants grown in space might simply be artifacts due to some unrecognized difference between the space plants and their earth-bound controls. The uncertainties of each spaceflight make it impossible to have completely effective ground control. This problem can be overcome only be having an on-board 1-g centrifuge to provide the necessary control. In addition, there are many experiments in which g-forces between 0 and 1 should be tried. This can best be done by having the on-board centrifuge. The disadvantage of this setup is that the radius of the centrifuge would be so small that only small plants or tissue cultures (or cell suspensions) could be used in this chamber. However, the opportunity to do a number of experiments each year by using a mid-deck locker far outweigh the disadvantages of the restriction to small plants.
- The final, essential plant growth unit would be a lighted plant environmental unit (PEU). The unit should be capable of having illumination of at least 300 umoles/sec m², with short periods of illumination of up to 1000 umoles/sec m^2 . The illumination should be cool white fluorescent or equivalent. Temperature should be capable of being held to $\pm 1^{\circ}$ C, with a range of 20-40° C in the light and 15-40° C in the dark. CO2 needs to be controlled carefully, with a range from 350 to 3000 ppm available. Relative humidity should be between 50 and 85%, with it held +5%. The air would probably be a flow-through system, with good air mixing in the chambers produced by fans. Removal of unwanted volatiles would again be required. An important difference in this unit would be the ability to monitor CO2, H2O, ethylene, H2S and SO2 with a gas chromatographic setup. Ideally this unit should also contain an on-board centrifuge. It is desirable, but of lower priority, for there to be some system for measuring the uptake of certain nutrients to the roots. It is recognized that the information is not available as to the best method for supplying water and nutrients to the roots, and the possibility of further engineering tests in space to solve this problem are indicated. The unit should have radiation monitoring equipment (if not available elsewhere in the space-lab) and vibration monitoring equipment. It is anticipated that this unit would be the major

plant unit for space-lab, but since the number of available flights on which this unit could be used is far less than those in which PGU or PGU-2 might be used, preliminary and exploratory experiments should be designed to use PGU or PGU-2 rather than the PEU. The group was very optimistic that if these pieces of hardware were available, there should be a large number of plant scientists desiring to make use of them to take advantage of the opportunity of microgravity to answer important botanical questions.

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